Rapid renewal of auditory hair bundles

The recovery time after noise-induced hearing loss is in step with a molecular treadmill.

Stereocilia, also known as hair bundles, are mechanosensitive organelles of the sensory hair cells of the inner ear that can detect displacements on a nanometre scale and are supported by a rigid, dense core of actin filaments. Here we show that these actin-filament arrays are continuously remodelled by the addition of actin monomers to the stereocilium tips, and that the entire core of the stereocilium is renewed every 48 hours. This unexpected dynamic feature of stereocilia will help our understanding of how auditory sensory function develops and is maintained.

Stereocilia develop before or immediately after birth according to a hair-cell developmental programme that generates a 'staircase' bundle of stereocilia (Fig. 1a) of precise number and height[1], and which grow at about 0.5 μm per day[1]. However, the molecular mechanisms by which stereocilia are formed and maintained are poorly defined[1,2].

To determine the locus of actin polymerization during stereocilium development and the degree of actin turnover after the stereocilia are fully formed, we exploited the preferential localization of the β-isoform of actin in stereocilia[3] (Fig. 1b). We used stereocilia from hair cells of the organ of Corti from newborn rats, which can be maintained in culture for up to 15 days[4,5]. During this time they develop a complex structural[4] and functional[5] phenotype that is similar to that of their *in vivo* counterpart.

We monitored the transient expression of β-actin and its incorporation into actin filaments in stereocilia after transfecting these cultures with a plasmid DNA construct encoding β-actin and enhanced green fluorescent protein (actin–GFP). We used a 'gene-gun' system to transfect segments from the middle turn of the organ of Corti that were dissected at postnatal day 1–3 and maintained in culture. We fixed cultures at various times after transfection and counterstained the total actin with rhodamine–phalloidin before viewing samples through a confocal microscope.

Hair cells rapidly and progressively incorporated actin–GFP, which became visible at the tips of stereocilia within 4 h of transfection and then progressed towards the base. In young (postnatal days 3–5) cultures ($n = 27$), actin–GFP was evident along the entire length of the stereocilia as early as 6 h after transfection (Fig. 1c). From this observation, we calculate that actin filaments polymerize at a rate that is about 50 times faster than was previously reported[1].

In our 10–15-day-old cultures, the presumably mature bundles[6,7] continued to incorporate actin–GFP from tip to base until the entire length of the stereocilium was labelled (after 48 h; Fig. 1d). Incorporation was progressive and uniform in these fully developed stereocilia, indicating that each entire actin-filament bundle treadmills towards the base at a rate of 2.5 μm per day (Fig. 1) as new actin monomers are incorporated into the filaments at the tip.

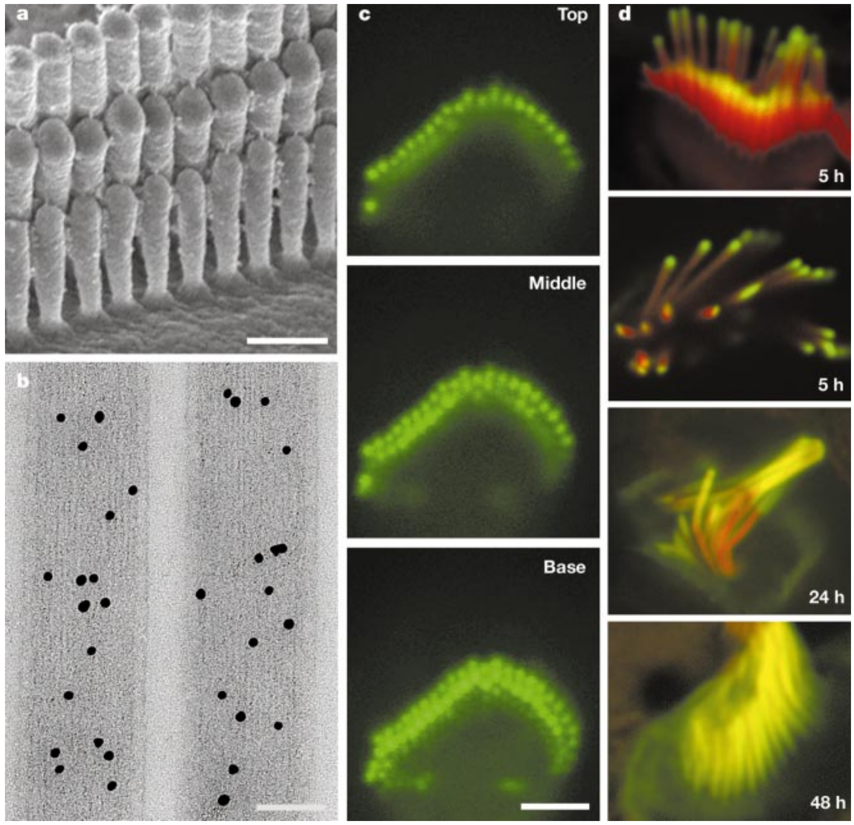

Figure 1 Continuous renewal of actin in auditory stereocilia. **a, b,** Scanning (**a**) and thin-section (**b**) electron micrographs of stereocilia, processed as described[10]. Immunogold labelling (**b**) reveals the homogeneous distribution of native β-actin along the semicrystalline array of actin filaments in two stereocilia. **c, d,** Fluorescence confocal images of actin–GFP incorporation (green) in stereocilia of rat hair cells from cultured organ of Corti. **c,** Stereocilia bundle from a postnatal-day-5 culture, 5 h after transfection; cross-sections of the top, middle and base of the bundle show incorporation along the length of the three rows of stereocilia. **d,** Double-labelled images of stereocilia from 10–15-day-old cultures at the indicated times after transfection. Actin filaments are stained red; regions of overlap between red-stained actin and newly incorporated β-actin–GFP appear yellow. Incorporation begins at the tip, has progressed halfway down the stereocilium after 24 h, and has reached the base after 48 h. The progression rate is 2.5 ± 0.18 μm per day ($n = 127$; calculated from the length of the GFP-labelled region in the tallest row of stereocilia from hair cells ($n = 30$) transfected for 24 or 48 h). Scale bars, 1 μm (**a**), 0.1 μm (**b**) and 2 μm (**c**, **d**).

This rapid, systematic incorporation of actin monomers at the tips of stereocilia in mature hair bundles is evidence of a previously unknown, continuous renewal process that is analogous to the migration and renewal of the photoreceptor discs in the retina[8]. The turnover rate of 48 h for this process in mature bundles is of the same order as the recovery time from noise-induced temporary hearing loss[9], indicating that the core structure of stereocilia may play an unforeseen part in this recovery. Our discovery that the renewal of stereocilia is dynamic may be of value in the investigation of conditions associated with malformation or disruption of stereocilia.

Mark E. Schneider, Inna A. Belyantseva, Ricardo B. Azevedo, Bechara Kachar

Section on Structural Cell Biology, National Institute on Deafness and Other Communication Disorders, National Institutes of Health, Bethesda, Maryland 20892-4027, USA

e-mail: kacharb@nidcd.nih.gov

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Artificial intelligence

Fast hands-free writing by gaze direction

Here we describe a method for text entry based on inverse arithmetic coding that relies on gaze direction alone and which is faster and more accurate than using an on-screen keyboard. These benefits are derived from two innovations: the writing task is matched to the capabilities of the eye, and a language model is used to make predictable words and phrases easier to write.

For people who cannot use a standard keyboard or mouse, the direction of gaze is one of the few means by which they can convey information to a computer. Many systems for gaze-controlled text entry provide an on-screen keyboard with buttons that can be 'pressed' by staring at them. But eyes did not evolve to push buttons, and this method of writing is exhausting.

Moreover, on-screen keyboards are inefficient because typical text has considerable redundancy[1]. Although a partial solution to this defect is to include word-completion buttons as alternative buttons alongside the keyboard, a language model's predictions can be better integrated into the writing process. By inverting an efficient method for text compression — arithmetic coding[2] — we have created an efficient method for text entry, which is also well matched to the eye's natural talent for search and navigation.

One way to write a piece of text is to delve into a theoretical 'library' that contains all possible books, and find the book that contains exactly the desired piece of text[3]; writing thus becomes a navigational task. In our idealized library, the 'books' are arranged alphabetically on one enormous shelf. As soon as the user looks at a part of the shelf, the view zooms in continuously on the point of gaze. So, to write a message that begins "hello", the user first steers towards the section of the shelf marked 'h', where all the books beginning with 'h' are found. Within this section are different sections for books beginning 'ha', 'hb', 'hc' and so on; the user enters the 'he' section, then the 'hel' section within it, and so forth.

To make the writing process efficient, we use a language model, which predicts the probability of each letter's occurrence in a given context, to allocate the shelf space for each letter of the alphabet (Fig. 1a). When the language model's predictions are accurate, many successive characters can be selected by a single gesture.

We previously evaluated this system, which we call 'Dasher', with a mouse as the steering device[4]. Novices rapidly learned to write and an expert could write at 34 words per minute; all users made fewer errors than when they were using a standard 'QWERTY' keyboard.

Figure 1b shows an evaluation of Dasher driven by an eye-tracker, compared with an on-screen keyboard. After an hour of practice, Dasher users could write at up to 25 words per minute, whereas on-screen keyboard users could manage only 15 words per minute. Moreover, the error rate with the on-screen keyboard was about five times that obtained with Dasher.

Users of both systems reported that the on-screen keyboard was more stressful to use than Dasher for two reasons. First, they often felt uncertain whether an error had been made in the current word (the word-completion feature works only if no error has been made); an error can be spotted only by looking away from the keyboard. Second, a decision has to be made after 'pressing' each character on whether to use word completion or to continue typing — looking to the word-completion area is a gamble as it is not guaranteed that the required word will be there, and finding the correct completion requires a switch to a new mental activity. By contrast, Dasher users can see simultaneously the last few characters they have written and the most probable options for the next few. Furthermore, Dasher makes no distinction between word completion and ordinary writing.

Dasher works in most languages — the language model can be trained on sample documents and adapts to the user's language as he or she writes. It can also be operated with other pointing devices, such as a touch screen or rollerball. Dasher is potentially an efficient, accurate and fun writing system not only for disabled computer users but also for users of mobile computers.

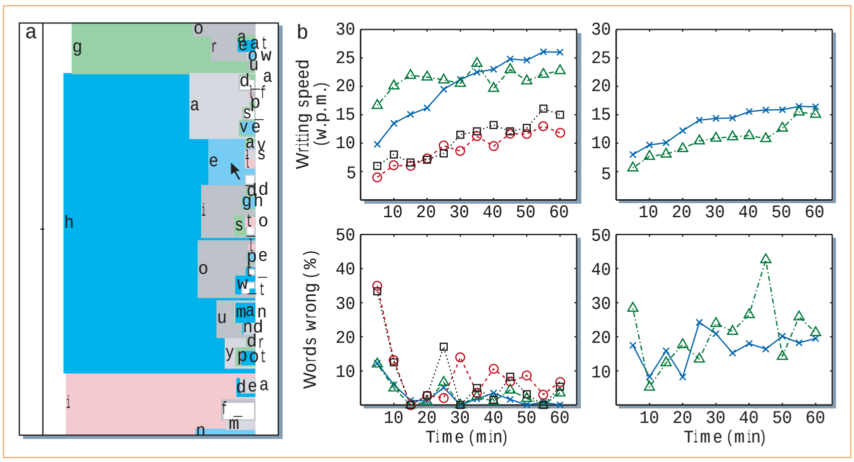

Figure 1 Hands-free text entry. **a,** Screenshot of 'Dasher'[5] when the user begins writing "hello". The shelf of the alphabetical 'library' is displayed vertically. The space character (represented as an underscore) is included in the alphabet after 'z'. In this example, the user has zoomed in on the portion of the shelf containing messages beginning with 'g', 'h' and 'i'. Following the letter 'h', the language model makes the vowels and 'y' easier to write by giving them more space. Common words such as 'had' and 'have' are visible. The arrow indicates the gaze of the user; its vertical coordinate controls the zooming-in point and its horizontal coordinate controls the rate of zooming; looking to the left makes the view zoom out, allowing recent errors to be corrected. **b,** Comparison of writing speeds and error rates for two methods of gaze-driven text entry. Left, Dasher with eye-tracker, as recorded for two expert users of the system (crosses, triangles) and two novices (circles, squares); right, on-screen keyboard, used by two experts on the 'QWERTY' keyboard. The eye-tracking system was EyeTech's Quick Glance eye-tracker. Each user took dictation from Jane Austen's *Emma* in 5-min sessions. The language model PPMD5 predicts the next character when given the previous five characters[6,7]; it was trained on passages from *Emma* not included in the dictation. Right panels, the two experts took dictation using the same eye-tracker to control the WiViK on-screen keyboard (a standard 'QWERTY' keyboard) with the word-completion buttons enabled.

David J. Ward, David J. C. MacKay
Cavendish Laboratory, Cambridge CB3 0HE, UK
e-mail: mackay@mrao.cam.ac.uk

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